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Immunology

Shared HLA class II-associated genetic susceptibility and resistance, related to the *HLA-DQB1* gene, in IgA deficiency and common variable immunodeficiency

(genetic linkage/HLA-DQ/HLA-DR/complement components C2 and C4/steroid 21-hydroxylase)

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ABSTRACT **Most cases of selective IgA deficiency (IgA-D) and common variable immunodeficiency (CVID) occur sporadically. However, familial clustering is not uncommon, and the two disorders can occur within the same family. We have previously described positive associations with three DR–DQ haplotypes as well as a strong negative association with DRw15,DQw6,Dw2 in IgA-D. Different amino acids at position 57 of the HLA-DQβ chain were found to be related to susceptibility and resistance to IgA-D. Now we have found identical, although somewhat weaker, positive and negative DR–DQ associations in a large group of CVID patients ($n = 86$), as well as the same associations with codon 57 of the *DQB1* gene. In addition, we have confirmed our earlier observations in an independent group of IgA-D individuals ($n = 69$), and in sib-pair analysis we have found linkage of the genetic susceptibility to IgA-D to the HLA class II region. In IgA-D individuals not carrying the three overrepresented DR–DQ haplotypes, the same positive association with a non-aspartic acid residue at position 57 of the HLA-DQβ chain was seen. The previously reported associations with deletions of the HLA class III genes *C4A* (fourth component of complement) and *CYP21P* (steroid 21-hydroxylase pseudogene) were, in our groups of immunodeficient individuals, statistically secondary to the association with the *DQB1* allele *0201*. The shared HLA class II associations in the two humoral immunodeficiencies support the hypothesis that IgA-D and CVID are related disorders. Disease susceptibility and resistance are most closely associated with a gene(s) within the DR–DQ region, alleles of the *DQB1* locus being candidate genes.**

Most expressed human leukocyte antigen (HLA) loci exhibit a remarkable degree of allelic polymorphism. Allelic HLA variability determines the immune response phenotype of an individual by influencing the self-adjustment of the T-cell repertoire during thymic maturation and the presentation of antigenic peptides. Furthermore, many diseases with autoimmune features are associated with HLA alleles. With the introduction of genomic typing techniques, a few of the HLA-associated diseases have been found to be related to specific amino acids or epitopes of the polymorphic membrane-distal domains of the class II molecules. This has been most convincingly shown in insulin-dependent diabetes mellitus (IDDM) (1–3) and immunoglobulin A deficiency (IgA-D) (4, 5), both diseases positively and negatively associated with the same amino acids at position 57 of the HLA-DQβ chain, and in rheumatoid arthritis (6, 7), associated with a conserved sequence motif of the *DRB1* gene.

Selective IgA-D and common variable immunodeficiency (CVID) are the two most prevalent immunodeficiencies in Caucasians. These heterogeneous disease entities are not always clearly separable. Thus, IgA-D individuals may also be deficient in one or several IgG subclasses. On the other hand, patients with CVID are usually panhypogammaglobulinemic, but they may have normal levels of IgM. CVID and IgA-D are clinically manifested in recurrent bacterial respiratory tract infections. Gastrointestinal disorders and diseases with autoimmune features are also common in these patients. In most individuals with IgA-D or CVID, normal numbers of B cells are detected, but they fail to undergo terminal differentiation into antibody-secreting cells (8, 9).

The familial predisposition to IgA-D and CVID suggests that genetic factors influence disease susceptibility. Evidence for linkage with the HLA region has been obtained in some (10, 11) but not all (12) segregation studies. In population studies of IgA-D, associations were first described with HLA class I alleles, most consistently with A1 and B8 (12–15) but also with A28 and B14 (15–17). Subsequently, an association with the HLA class II allele DR3 has been repeatedly observed (10, 14, 15, 18) and of late found to be with the DRw17 subtype of DR3 (4). Positive associations with DR1 (17) and DR7 (15, 18) as well as a negative DR2 association (18) have also been described. An insignificant increase of DR3 has recently been noted in CVID (19). Furthermore, similar associations with deletions and polymorphisms of HLA class III genes have been described in IgA-D and CVID, suggesting that the two disorders are related (20).

We have previously shown that selective IgA-D is positively associated with three DR–DQ haplotypes and is strongly negatively associated with a fourth haplotype (4). These associations were found to be related to the amino acid residue at position 57 of the DQβ chain, aspartic acid (Asp) being associated with resistance and non-aspartic acid (non-Asp) with susceptibility to disease. In a small number of patients with combined IgA-D and IgG2 subclass deficiency similar associations with DR–DQ haplotypes and Asp/non-Asp have subsequently been identified (5). In the present work, we were interested in investigating if similar HLA class II associations were to be found in patients with CVID and also in seeking confirmation of our previous findings in an independent group of individuals with IgA-D. HLA-DP typings and investigation of restriction fragment length polymorphisms (RFLPs) of HLA class III genes were also performed to delineate the associated haplotypes and by linkage calcu-

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Abbreviations: IgA-D, immunoglobulin A deficiency; CVID, common variable immunodeficiency; IDDM, insulin-dependent diabetes mellitus; Asp, HLA-DQβ chain with aspartic acid at position 57; non-Asp, HLA-DQβ chain with a neutral amino acid at position 57; RFLP, restriction fragment length polymorphism; C2, second component of complement; C4, fourth component of complement; RR, relative risk.

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lations identify the statistically most implicated part of the HLA region.

MATERIALS AND METHODS

Immunodeficient Individuals. One hundred and sixty-four unrelated Swedish IgA-D individuals as well as six disease-concordant siblings were studied. IgA-D was defined as a serum IgA of less than 0.05 g/liter. Approximately one-third of the IgA-D individuals had symptomatic IgA-D, in most cases manifested by recurrent sinopulmonary infections. The HLA class II alleles of 95 of the IgA-D individuals have been reported previously (4). Eighty-six unrelated Swedish patients with CVID were studied. The diagnosis of CVID was based on a typical history and decreased serum immunoglobulin levels; IgA $\leq$ 0.1 g/liter and IgG $\leq$ 4.2 g/liter. Serum IgM varied from <0.02 to 1.8 g/liter.

Controls. Two hundred and fifty randomly selected healthy Swedes were used as controls.

Southern Blot Analysis. DNA was isolated from peripheral blood leukocytes. Samples (8–10 μg) of DNA were digested with *Bam*HI, *Msp* I, or *Taq* I. Agarose gel electrophoresis, capillary blotting onto nylon membranes, labeling of purified probe inserts, hybridization, stringency washes, and autoradiography were performed according to standard techniques with minor modifications (21).

***Taq* I *DRB*, *DQA*, and *DQB* RFLP Typing.** Allelic *Taq* I *DRB*, *DQA*, and *DQB* restriction fragment patterns were analyzed as previously described (22, 23). The serologically defined DR and DQ specificities as well as the cellularly defined Dw specificities associated with the different *Taq* I *DRB–DQA–DQB* haplotypes are given in the text (22, 23).

***Msp* I *DPA* and *DPB* RFLP Typing.** Allelic *Msp* I *DPA* and *DPB* restriction fragment patterns were analyzed as described in ref. 24. The cellularly defined DP specificities associated with the different allelic *Msp* I patterns are given in the text (24).

***In Vitro* Amplification of *DRB1* Genes in DR4 Haplotypes.** The second exon of the *DRB1*04* genes was amplified by group-specific polymerase chain reaction (PCR) (36) in 26 IgA-D and 27 CVID patients and in 69 controls. Seven DR4-positive homozygous cell lines were included as amplification and hybridization controls.

***In Vitro* Amplification of *DQB* Genes.** The polymorphic second exon of the *DQB* genes was PCR amplified (1) in 72 IgA-D individuals and 100 controls. Twenty-six homozygous cell lines representing 13 *DQB1* alleles were used as specificity controls.

Hybridization with Oligonucleotide Probes. The PCR products were dot blotted onto nylon membranes; 3′-end-labeling of oligonucleotide probes with ^{32}P, hybridization, and stringency washes were performed as described in ref. 3. The sequences of the oligonucleotides used for DR4 subtyping and for *DQB1* typing are given in refs. 1 and 3, respectively.

***Bam*HI RFLPs of the *C2* Gene.** *Bam*HI-cleaved DNA was hybridized with a 2-kilobase (kb) *Eco*RI–*Eco*RI cDNA fragment of the gene encoding the second component of complement (*C2*) (25) in 54 IgA-D and 38 CVID patients and in 100 controls. Five allelic *C2* *Bam*HI fragments have been described: 3.20, 3.30, 3.35, 3.40, and 3.45 kb (20).

Deletion of the *C4A* Gene. Deletions of the gene encoding the fourth component of complement (*C4A*) were detected by hybridizing *Taq* I-digested DNA with a 0.5-kb *Bam*HI–*Kpn* I *C4* cDNA fragment (26). Deletion of the *C4A* gene is in most cases associated with the appearance of a 6.4-kb band (27).

Deletion of the *CYP21P* Gene. Deletions of the steroid 21-hydroxylase pseudogene (*CYP21P*) were determined by densitometric scanning of *Taq* I-cleaved DNA hybridized with a near full-length cDNA probe (28).

Statistical Analysis. Data were analyzed by the χ^2 test, or in case of small numbers, Fisher's exact test. *P* values were not corrected for multiple comparisons when our previously described DR–DQ associations were investigated in a new group of IgA-D individuals compared to a new group of controls or when the same associations were investigated in patients with CVID. Strength of associations is given as relative risk (RR). Two-locus linkage analyses were performed as described in ref. 29.

RESULTS

Genetic Linkage. Linkage of the genetic susceptibility to IgA-D to the HLA region was ascertained in sib-pair analysis. Four of six disease-concordant sib pairs were DR–DQ identical and two were haploidentical ($P < 0.05$).

DR–DQ Haplotypes. We have previously identified positive associations with the DR1,DQw5, DR7,DQw2, and DRw17,DQw2 haplotypes as well as a strong negative association with DRw15,DQw6,Dw2 in IgA-D (4). In the new group of individuals with IgA-D compared with a new group of controls the same positive and negative associations were found (Table 1). In addition to being seen in two independent studies, these four associations were all significant after correction for multiple comparisons. It is of interest to note that the two DR–DQ haplotypes (DRw8,DQw4 and DRw13,DQw6,Dw18) and the DQw7 allele with neutral to negative associations in our first IgA-D study were also underrepresented in the second study, two of them significantly ($P < 0.05$). However, although these associations were found in both studies, they were not strong enough to withstand correction for multiple comparisons.

In Caucasians, DR4 and DR7 are the only two commonly occurring DR specificities that are associated with more than one DQ allele. Most DR4 haplotypes in IgA-D individuals were DQw8-positive ($P < 0.05$) (Table 1). However, the distribution of the DR4-associated cellular specificities was similar in DR4-positive IgA-D patients and controls. Significantly more DR7 haplotypes in IgA-D patients were DQw2-positive and not DQw9-positive ($P < 0.05$) (Table 1).

In CVID the same, but somewhat weaker, positive and negative DR-DQ associations as in IgA-D were found (Table 1). All these associations, except the association with the DR7,DQw2 haplotype, were still significant after correction for multiple comparisons.

In CVID no altered distribution of DQ alleles in DR4-positive patients and only an insignificant tendency of DR7-positive haplotypes being DQw2-positive ($P < 0.06$) was observed (Table 1). The frequencies of the DR4-associated cellular subtypes were similar in CVID and controls.

In IgA-D we have previously shown that the RRs conferred by the DR7,DQw2 and DRw17,DQw2 haplotypes were additive, whereas DR1,DQw5 acted synergistically with these two haplotypes (4). In the two combined IgA-D studies most combinations of hetero- or homozygosity for the associated DR–DQ haplotypes were synergistic, except DR1,DQw5/DRw17,DQw2 heterozygosity and DR1,DQw5 homozygosity, with RRs from 12.5 to 20.2. Homozygosity for the *DQB1*0201* allele conferred the highest RR, independent of the individual's being DRw7,DQw2 or DR17,DQw2 homozygous or DR7,DQw2/DRw17,DQw2 heterozygous (RR = 12.5–22.6). However, in CVID the RRs of most combinations of the positively associated DR–DQ haplotypes were strictly additive, except DR1,DQw5/DR7,DQw2 heterozygosity and DR7,DQw2 homozygosity. Eighty-three percent of IgA-D individuals and 71% of CVID patients carried one or more of the associated haplotypes, compared with 39% of the controls ($P < 10^{-10}$, RR = 7.7, and $P < 10^{-6}$, RR = 3.8, respectively).

Amino Acids at Position 57 of the HLA-DQβ Chain. When looking for a shared feature between the positive, negative,

Table 1. Frequencies of DR–DQ phenotypes and the DQw2 and DQw7 alleles in patients with IgA-D or CVID and in healthy controls

			Frequency, %					Frequency, %		
			First group		Second group					
DR	DQ	Dw	IgA-D (n = 95)	Controls (n = 100)	IgA-D (n = 69)	Controls (n = 150)	RR†	CVID (n = 86)	All controls (n = 250)	RR†
1‡	w5	1	37****	21	23*	16	2.1	37****	18	2.7
4	w7		3	11	4	13		14	12	
4	w8		21	24	23	27		27	26	
7§	w9	11	5	4	6	5		9	5	
7	w2	17	21***	7	22****	7	3.7	12*	7	1.8
w8	w4		2*	9	1*	9	(0.2)¶	6	9	
9§	w9		3	4	3	5		3	4	
w10	w5		2	3	1	1		0	2	
w11	w7		3	12	6	15		5	14	
w12	w7		4	6	6	6		9	6	
w13	w6	18	8*	22	6	15	(0.4)¶	13	18	
w13	w6	19	8	11	16	11		9	11	
w13	w7		1	1	3	2		2	2	
w14	w5	9	6	5	6	7		3	6	
w15	w6	2	3****	30	4***	30	0.1	7***	30	0.2
w16	w5	21	2	2	0	1		0	1	
w17	w2	3	46****	15	48****	19	4.3	33**	17	2.3
—	w2	—	59****	21	61****	25	5.0	43***	23	2.5
—	w7	—	12*	29	17*	34	(0.3)¶	27	32	

When looking for HLA class II associations other than the well-established DRw17 (3) association (10, 14, 15, 18), gene frequencies of patients and controls were compared after subtraction of DRw17,DQw2-positive haplotypes. The first group of IgA-D individuals was compared with the first group of controls (part of these data is from ref. 4), whereas the second independent group of IgA-D individuals was compared with a new group of randomly selected controls. CVID patients were compared with the combined two groups of controls. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

†When RRs were calculated, all the IgA-D (n = 164) and the CVID individuals were compared with all the controls.

‡DR1 and DR'Br' cannot be distinguished by *Taq* I RFLP typing (23). Eighteen IgA-D individuals, 12 CVID patients, and 25 controls carrying the DR1/DR'Br'-associated RFLP patterns were *DRB1*01* subtyped with allele-specific PCR amplification (30). All were DR1,Dw1-positive.

§DR7,DQw9 and DR9,DQw9 cannot be discriminated by *Taq* I RFLP analysis (23). All immunodeficiency patients and controls carrying the DR7,DQw9/DR9,DQw9 *Taq* I *DRB–DQA–DQB*-associated haplotype were typed by allele-specific PCR amplification (H. Zetterquist and O.O., unpublished results).

¶Neutral/negative association; see text.

and neutral/negative DR–DQ associations in our previous study, we found that aspartic acid at position 57 of the HLA-DQβ chain was associated with resistance to IgA-D, whereas a neutral amino acid at the same position was associated with disease susceptibility (4). The same associations were found in the new group of IgA-D patients. Fifty-nine percent of these patients and 17% of the new controls were DQβ non-Asp homozygous ($P < 10^{-8}$, RR = 7.0). Only 6% of the patients were Asp homozygous, whereas 31% of the controls were ($P < 10^{-4}$, RR = 0.1). The results of the two combined studies are shown in Fig. 1.

The same, but not as pronounced, associations with the amino acids at position 57 of the DQβ chain were found in CVID. Thirty-seven percent of the patients and 19% of all the controls were non-Asp homozygous ($P < 0.001$, RR = 2.5). Merely 10% of the CVID patients were HLA-DQβ Asp homozygous, whereas 32% of all the controls were ($P < 0.0005$, RR = 0.2) (Fig. 1).

When DR1,DQw5-, DR7,DQw2-, and DRw17,DQw2-negative IgA-D individuals were analyzed alone, it was found that the amino acids at position 57 were associated with disease susceptibility and resistance in this subgroup also ($P < 0.02$) (Table 2). The same result was obtained when haplotypes negative for the three positive DR–DQ associations were examined ($P < 0.001$). Similar, but statistically insignificant, trends were found in CVID in both kinds of analyses (Table 2).

DP Alleles. As expected, an increase of the DPw1 allele, part of the A1,B8,DRw17,DQw2,DPw1 extended haplotype, was seen mainly in IgA-D but also in CVID. However, after correction for multiple comparisons no statistically significant DP association was found in either disorder (Table 3).

***Bam*HI *C2* RFLPs.** There was no difference in the occurrence of the common *Bam*HI *C2* 3.4-kb fragment in immunodeficiency patients compared with controls. The combined frequencies of rare *C2* RFLPs—i.e., 3.20-, 3.30-, 3.35-, and 3.45-kb bands—were similar in IgA-D, CVID, and controls (19%, 14%, and 16%, respectively).

***C4A* Gene Deletions.** In IgA-D a strong association with deletion of the *C4A* gene was observed: 47% of the patients,

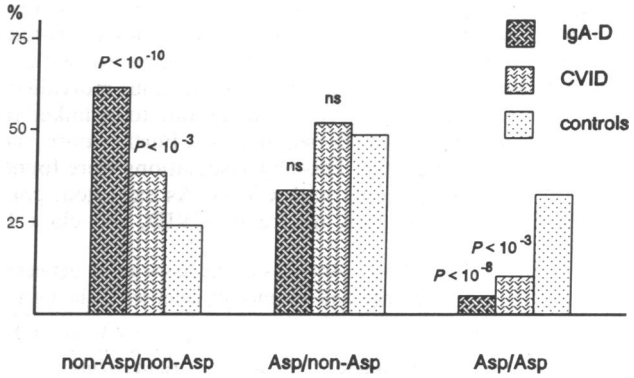

FIG. 1. The frequencies (%) of homo- and heterozygosity for HLA-DQβ chains carrying non-Asp or Asp at position 57 in individuals with IgA-D or CVID compared with healthy controls. All the patients with IgA-D (n = 164) or CVID (n = 86) and all the controls (n = 250) were HLA-DR and -DQ typed by *Taq* I RFLP analysis (22, 23) and the amino acid at position 57 was inferred. In addition, codon 57 of the *DQB1* genes was directly determined by PCR–sequence-specific oligonucleotide typing (1, 3) in 72 of the IgA-D individuals, 100 of the controls, and 26 homozygous cell lines. The two methods gave completely concordant results.

Table 2. Distribution of non-Asp/non-Asp, non-Asp/Asp, and Asp/Asp genotypes in DR1,DQw5-negative, DR7,DQw2-negative, and DRw17,DQw2-negative individuals with IgA-D or CVID and in DR1,DQw5-, DR7,DQw2-, and DRw17,DQw2-negative healthy controls

Individuals	*n*	Frequency, % non-Asp/non-Asp	non-Asp/Asp	Asp/Asp	*P*
IgA-D	28	21	50	29	<0.02
CVID	25	16	48	36	<0.06
Controls	153	9	38	53	

compared with 17% of the controls ($P < 10^{-10}$, RR = 4.4). In CVID the association was weaker: 30% of the patients ($P < 0.01$, RR = 2.1). *C4A* deletions were almost exclusively observed in DRw17,DQw2-positive individuals, 95% of the patients and 88% of the controls. Furthermore, all but one of the DR17,DQw2 homozygous individuals, patients and controls, had homozygous *C4A* deletions. As two of the DR–DQ haplotypes positively associated with IgA-D and CVID (DR7,DQw2 and DRw17,DQw2) have identical DQβ chains (*DQB1*0201*), this shared feature can be used as one marker in two-locus linkage analysis. As shown in Table 4, the association with the *DQB1*0201* allele was primary in both IgA-D and CVID ($P < 10^{-5}$ and $P < 0.05$, respectively).

***CYP21P* Gene Deletions.** In IgA-D a strong association with deletion of *CYP21P* gene was observed: 43% of the patients but only 18% of the controls ($P < 10^{-7}$, RR = 3.4). In CVID the association was weaker: 31% of the patients ($P < 0.05$; RR = 2.0). *CYP21P* deletions were predominantly seen in DRw17,DQw2-positive individuals, 87% of the patients and 81% of the controls. Furthermore, all but one of the DR17,DQw2 homozygous individuals, patients and controls, had homozygous *CYP21P* deletions. Linkage calculations showed that the *CYP21P* association was secondary to the primary *DQB1*0201* association in both IgA-D and CVID ($P < 10^{-7}$ and $P < 0.05$, respectively) (Table 4).

DISCUSSION

In two independent studies of HLA class II associations in IgA-D, we have observed strikingly similar positive, negative, and neutral/negative associations. The disease susceptibility conferred by the associated DR–DQ haplotypes acted synergistically. Thus, a person carrying two of the associated haplotypes or two non-Asp DQβ chains had a 100- to 200-fold increased risk of developing IgA-D compared with a DRw15,DQw6-positive or DQβ Asp homozygous individual. The genetic susceptibility to IgA-D was found to be linked to the HLA class II region in sib-pair analysis. Furthermore, the same positive and negative DR–DQ associations were found in a large group of patients with CVID. As expected, considering the heterogeneous nature of CVID, the class II associations were weaker in this group. The same DR–DQ associations were seen in CVID patients irrespective of the IgM level or degree of IgA-D. The shared HLA class II-associated genetic susceptibility and resistance strongly favor the hypothesis that IgA-D and a large, as-yet-uncharacterized, subgroup of CVID are related. The theory is further supported by the similar clinical findings in these patients, by the co-occurrence of the two conditions in the same families, and by anecdotal reports of patients showing a gradual shift from IgA-D to CVID.

Table 3. Distribution of DP alleles in patients with IgA-D or CVID and in healthy controls

DP allele	Frequency, % IgA-D (*n* = 69)	CVID (*n* = 53)	Controls (*n* = 250)
w1	22*	17*	8
w2	17	8	21
w3/w6†	35	51	36
w4	83	74	86
w5	7	6	6
CDP HEI	4	2	2

*IgA-D, $P < 0.005$ and *P* corrected not significant. CVID, $P < 0.05$ and *P* corrected not significant.
†DPw3 and DPw6 cannot be separated by RFLP analysis (24).

The lack of association with DP and *Taq* I *DQA2* alleles, as well as the observation that the linkage disequilibrium between DRw17,DQw2 and DPw1 and between the three positively associated DR–DQ haplotypes and *DQA2* alleles is not more pronounced in IgA-D or CVID than in controls, shows that the candidate gene(s) most likely is located telomeric to the *DQA2* locus. The associations with loci in the HLA class III region in both IgA-D and CVID and with HLA class I alleles in IgA-D indicates that the immunodeficiency-associated haplotypes are extended telomerically, in IgA-D as far as into the HLA class I region.

Susceptibility to IgA-D has been suggested to be associated with ancestral HLA haplotypes (10) or with central HLA genes (31). Furthermore, similar associations with rare *Bam*HI *C2* RFLPs and deletions of *C4A* and *CYP21P* have been described in IgA-D and CVID, linking the two disorders and further implicating the HLA class III region (20). In the present study an increased occurrence of rare *Bam*HI *C2* RFLPs was not confirmed. Associations with deletions of *C4A* and *CYP21P* were found especially in IgA-D but also in CVID. However, the associations with *C4A* and *CYP21P* gene deletions in both IgA-D and CVID were statistically secondary to the association with the *DQB1*0201* allele.

The tight linkage disequilibrium between the DR and DQ subregions makes it difficult to determine the primary association(s) within the disease-associated DR–DQ haplotypes. However, the positive association with *DQB1*0201* and the neutral/negative association with DQw7 in IgA-D, as well as the skewed distribution of DQ alleles in DR4- and DR7-

Table 4. Analysis of the association between IgA-D and CVID and the *DQB1*0201* allele in the presence and absence of deletion of the *C4A* or *CYP21P* gene

Individuals	*n*	*C4A* del⁺/*CYP21P* del⁺ *DQB1*0201*⁺	*C4A* del⁺/*CYP21P* del⁺ *DQB1*0201*⁻	*C4A* del⁻/*CYP21P* del⁻ *DQB1*0201*⁺	*C4A* del⁻/*CYP21P* del⁻ *DQB1*0201*⁻
IgA-D	166	74/66	3/4	26/34	60/59
CVID	86	25/25	1/2	12/12	48/47
Controls	250	38/35	4/11	20/23	188/181

C4A deletion versus *DQB1*0201*:
Primary association with *DQB1*0201*: IgA-D, $\chi^2 = 19.1$; CVID, $\chi^2 = 5.1$.
Primary association with *C4A* deletion: IgA-D, $\chi^2 = 2.7$; CVID, $\chi^2 = 0.1$.
CYP21P deletion versus *DQB1*0201*:
Primary association with *DQB1*0201*: IgA-D, $\chi^2 = 31.0$; CVID, $\chi^2 = 5.5$.
Primary association with *CYP21P* deletion: IgA-D, $\chi^2 = 0.5$; CVID, $\chi^2 = 0.2$.

positive IgA-D individuals, in combination with a normal distribution of the DR4-associated cellular specificities, point towards the DQ subregion. In DR7,DQw2 and DRw17,DQw2 the primary association might be with the *DQB1* allele, as this is the same in the two haplotypes. The association is not with the specific DQ α-β heterodimer previously found to be associated with celiac disease (32), encoded in cis in DRw17,DQw2 and in trans in DR7,DQw2/DR5,DQw7 heterozygotes, as there was no overrepresentation of DR5,DQw7 in DR7,DQw2-positive immunodeficiency patients (2 of 44 patients, 3 of 17 controls) (Fig. 2). The *DQA1* allele of the negatively associated DRw15,DQw6 haplotype is also carried by the neutrally associated DRw13,Dw19. However, the *DQB1* alleles of the two negatively associated DRw15,DQw6 and DRw13,Dw18 haplotypes differ by only a single amino acid (Fig. 2). These data strongly indicate that both susceptibility and resistance to IgA-D and CVID are most closely associated with alleles of the *DQB1* gene. We favor the hypothesis that the HLA class II molecules themselves are involved in the pathogenesis of IgA-D and CVID. The mechanism(s) for this influence is not known, but it might operate via autoreactive T cells or via insufficient help from class II-restricted $CD4^+$ lymphocytes in T-cell-dependent B-cell responses, possibly due to inadequate response to infectious agents.

Position 57 of the HLA-DQβ chain has been shown to be an informative marker for IDDM in Caucasians and Negroes (refs. 1 and 2, reviewed in ref. 33). We have now confirmed the association with residue 57 in IgA-D and also found that CVID is associated with the same amino acid position. The shared associations with position 57 and the co-occurrence of IDDM and IgA-D found in a few studies (13, 34, 35), might be interpreted as a shared genetic predisposition. However, considering that the HLA class II allelic associations partly differ in IDDM and IgA-D/CVID and that an increased prevalence of IDDM was not found in our patients with IgA-D, unidentified disease-specific genetic and/or environmental factors may play a role in the pathogenesis.

In conclusion, our data indicate that IgA-D and CVID are genetically related disorders. Disease susceptibility/resistance is most closely associated with the DR–DQ region, alleles of the *DQB1* locus being candidate genes. In IgA-D and CVID, as in IDDM, the DR–DQ associations cannot merely be attributed to the charge of the amino acid at position 57. However, the structural and functional importance of this residue supports the hypothesis that the HLA class II molecules themselves might be involved in the pathogenesis and not only represent linked genetic markers. This view does not exclude the possibility that other genes within or outside the HLA region as well as environmental factors might influence disease susceptibility.

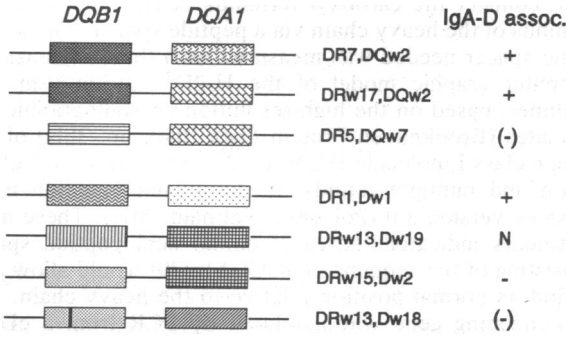

FIG. 2. Graphic comparison of *DQA1* and *DQB1* alleles of the IgA-D-associated DR–DQ haplotypes. +, positive association; −, negative; (−), neutral/negative; and N, neutral. Identical alleles have the same filling pattern. The *DQB1* alleles of DRw15,Dw2 and DRw13,Dw18 differ by a single amino acid substitution at position 30, tyrosine to histidine, indicated by a vertical bar.

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